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Report

Blue and Yellow Signal Cleaning Behavior in Coral Reef Fishes

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Summary

Marine cleaning symbioses are classic examples of mutualism: cleaners remove and consume ectoparasites from "client" fish, while clients benefit from a reduction in ectoparasites [1, 2]. However, how clients recognize cleaners and decide not to eat them is unclear. Color and body pattern are thought to be important in signaling cleaning services to coral reef fish [1, 3, 4]; in this study, we tested the long-held belief that cleaner fish display a blue "guild" coloration [5–7]. Via color analytical techniques and phylogenetic comparisons, we show that cleaner fish are more likely to display a blue coloration, in addition to a yellow coloration, compared to noncleaner fish. Via theoretical vision models, we show that, from the perspective of potential signal receivers, blue is the most spectrally contrasting color against coral reef backgrounds, whereas yellow is most contrasting against blue water backgrounds or against black lateral stripes. Finally, behavioral experiments confirm that blue within the cleaner fish pattern attracts more client reef fish to cleaning stations. Cleaner fish have evolved some of the most conspicuous combinations of colors and patterns in the marine environment, and this is likely to underpin the success of the cleaner-client relationship on the reef.

Results and Discussion

Do Cleaner Fish Exhibit a Blue "Guild" Coloration?

We measured the spectral reflectance of seven species of obligate cleaner fish and eleven species of facultative cleaner fish from the families Labridae and Gobiidae (Figure 1; see also Table S1 available online). Obligate cleaner fish are defined as cleaners that derive all of their dietary requirements from client-gleaned food, whereas facultative cleaner fish rely on other food sources, such as demersal eggs or phytoplankton [1]. Where possible, both juveniles and adults of the same species that exhibited different body patterns and/or cleaning behavior were measured. We then compared the spectral reflectance of cleaner fish to 31 noncleaning species from the Labridae family whose colors have been measured previously [8] (Figure 1; Table S1). This family was chosen because it encompasses the majority of obligate cleaner fish, plus many facultative cleaners.

Colors were defined and categorized by the shape of their spectrum (as per [8]) to provide a nonsubjective analysis of color and a convenient description that we can easily understand [8, 9]. Blue/Red is defined as having a peak in reflectance at 450–500 nm and a step in reflectance at 650–700 nm; UV/Blue is a bell-shaped curve with wavelengths in both the ultraviolet (<400 nm) and blue regions of the spectrum. Both of these colors can appear dark blue to turquoise to the human eye. Yellow has a step in reflectance at around 500 nm; UV/Yellow has an additional peak in the UV. Black has a low reflectance over the entire spectrum. For clarity, color names with a capital letter represent color categories [8], whereas colors with a lowercase letter represent a subjective description of a range of colors. For example, Blue refers to the color category defined as a bell-shaped curve with reflectance only in the 400–500 nm region of the spectrum, whereas blue refers to numerous variations in spectral shape, which can include colors from the UV/Blue, Blue/Red, and Blue categories, all of which appear blue to the human visual system.

We found that the color categories Blue/Red, UV/Blue, Yellow, and/or UV/Yellow occurred on all obligate and facultative cleaner fish (10 of 10 and 13 of 13, respectively) compared to 20 of 31 noncleaners (65%); Figure 2; Table S1). To investigate whether a blue or yellow coloration had evolved with cleaning traits, we used phylogenetic analysis with the program BayesTraits (www.evolution.rdg.ac.uk) to compare cleaning behavior with (1) the presence of Blue/Red, UV/Blue, Yellow, or UV/Yellow on the fish, (2) the presence of a lateral stripe of any color, or (3) the presence of a Black lateral stripe adjacent to a Blue/Red, UV/Blue, Yellow, and/or UV/Yellow color patch (or the converse). All cleaner fish (facultative and obligate) were significantly more likely to have a blue coloration (UV/Blue and Blue/Red combined) compared to noncleaner fish (likelihood ratio test statistic [LR] = 12.36, $p = 0.01$; Table S2). However, it appears that an overall blue coloration is important, rather than a particular category of blue, because individual categories (UV/Blue or Blue/Red) were not significant in our phylogenetic analysis (LR $\leq$ 8.38, $p > 0.07$; Table S2). Although adult cleaner fish displayed Blue/Red frequently (6 of 7 adult obligate cleaner species, with the exception of *Elacatinus figaro*), we suspect that the long-wavelength component of Blue/Red (>650 nm) is most likely beyond the spectral sensitivity range of our model fishes (Figure S1). Therefore, the long-wavelength component of Blue/Red probably has a nonadaptive function.

All cleaner fish were also more likely to have a Yellow coloration compared to noncleaner fish (LR = 17.88, $p < 0.01$; Table S2); however, this was not the case for UV/Yellow or combined yellow categories (Table S2). We did not find any significant results with color category for obligate cleaner fish, but this is probably a result of the fact that obligate cleaners are phylogenetically rare and there are only two obligate cleaning clades, restricting our sample size.

A dark lateral body stripe is considered to be a visual signal that advertises cleaning services in fish [1, 3, 4]; therefore, we also noted the presence or absence of one or more midlateral and/or dorsolateral contrasting stripes, which were found along more than half of the fishes' body length. Dorsal stripes were not taken into account. The color of the lateral stripe and the color of patches adjacent to the lateral stripe were also noted and measured. All of our obligate cleaner fish (10 of 10, 100%) had a lateral stripe that was either Black adjacent to Blue/Red, UV/Blue, Yellow, and/or UV/Yellow or, conversely,

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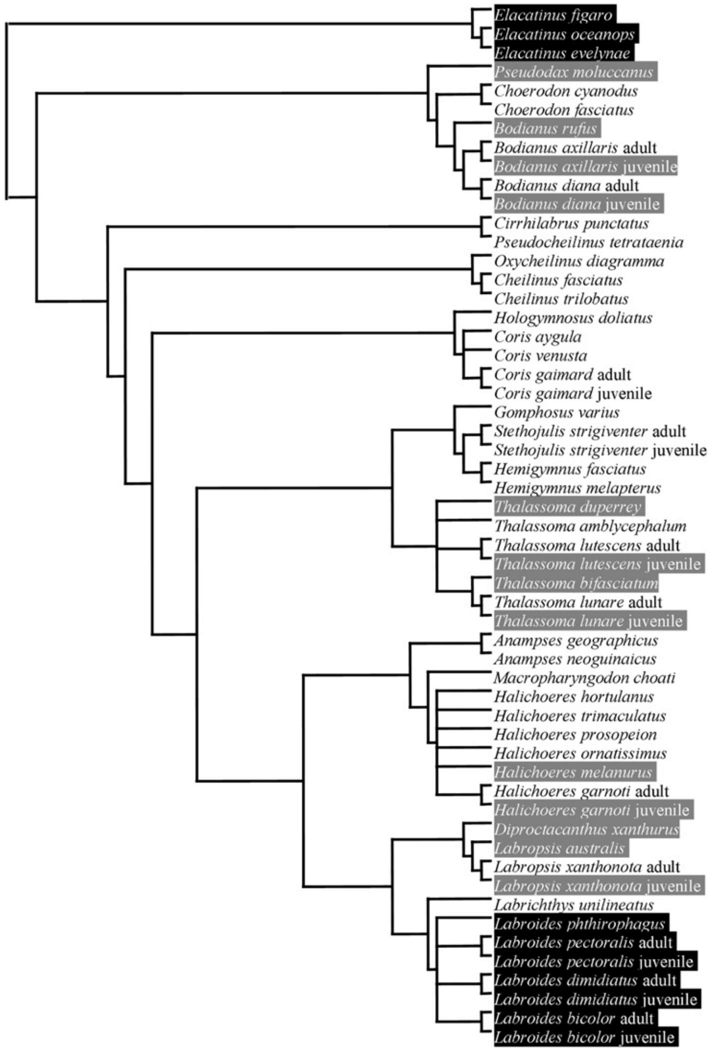

Figure 1. Phylogenetic Tree Obtained by Combining Phylogenies of Labridae and the Genus *Elacatinus*

Species highlighted in black are obligate cleaners; those highlighted in gray are facultative cleaners. Juveniles and adults of the same species that exhibit different body patterns and color signals were added as polytomies within species. For those species for which we collected color measurements that were not present in the original trees, we also added them as polytomies adjacent to other members of their genera. Labridae and *Elacatinus* phylogenies are from [32] and [24], respectively.

Blue/Red, UV/Blue, Yellow, and/or UV/Yellow adjacent to Black, compared to 2 of 13 facultative cleaners (17%) and 0 of 31 noncleaners (0%) (Figure 2; Table S1). All cleaner fish and obligate cleaners were significantly more likely to have a lateral body stripe compared to noncleaning fish (Table S2; also shown previously in [1, 3]). Also, this lateral stripe was more likely to be black adjacent to Blue/Red, UV/Blue, Yellow, and/or UV/Yellow color patches (or the converse) compared to any other color combination (Table S2).

Does Blue Make Cleaners More Conspicuous to Signal Receivers?

Cleaner wrasse, *Labroides dimidiatus*, interact with over 100 species of reef fish [10], and individuals of this species have been shown to clean an average of 2297 fish per day [11], including many large piscivores [12]. Therefore, we investigated how various client reef fish with different visual systems might view the colors and patterns of cleaner fish both empirically and behaviorally by (1) calculating the spectral contrasts within cleaner fish colors and against general background colors via theoretical vision models [13, 14] and (2) testing the response of wild client fish to seven fish models made from resin and painted with a range of colors and patterns.

Visual Modeling

We measured the visual response of three coral reef fish: the barracuda *Sphyraena helleri*, the UV-sensitive planktivorous damselfish *Abudefduf abdominalis*, and the herbivorous surgeonfish *Ctenochaetus strigosus*. These species were selected because their visual systems differ markedly, especially in their number of different types of cone photoreceptor [15] and in their sensitivity to UV wavelengths. In addition, they have all been observed to visit cleaning stations (K.L.C. and A.S.G., unpublished data), and they inhabit different niches of the coral reef [16].

For all three visual systems, blue categories (UV/Blue, Blue/Red, and Blue) were the most contrasting colors against an average coral background ($t > 2.14$, $p < 0.05$; Figure 3). UV/Blue and Blue/Red were significantly more contrasting than an average of the ten noncleaner colors combined (UV/Blue $t = 4.07$, $p < 0.001$; Blue/Red $t = 10.12$, $p < 0.001$; Figure 3). Therefore, a blue coloration, irrespective of color category, appears to be one of the most conspicuous colors when signaling to a variety of signal receivers against a coral reef background.

Blue is also an effective color for long-distance transmission in marine waters [17], and it is interesting that the category Blue appears not to be exhibited by cleaner organisms. Blue was more highly contrasting than UV/Blue or Blue/Red for *C. strigosus* and *S. helleri* against all three backgrounds ($p < 0.05$; Figure 3). The category of blue that is exhibited by cleaner fish may be limited by the physical structure of chromatophores, cells that contain pigments giving the fish its coloration [18].

Yellow was the most highly spectrally contrasting color against Black (lateral stripes on cleaner fish) and blue water backgrounds (Figure 3) and was significantly more contrasting than UV/Blue, Blue/Red, UV/Yellow, any other color category, or an average of the other ten colors combined (all $t > 2.01$, $p < 0.05$; Figure 3). Previous studies have shown that yellow is strongly contrasting against a blue water background [19–22]; however, we show here that a combination of colors within cleaner fish patterns is also highly contrasting. A combination of blue, yellow, and black enables cleaner fish to be highly visible when viewed from a number of directions and against different backgrounds. This is similar to the strawberry poison frog, *Dendrobates pumilio*, which exhibits extreme polymorphism in color and pattern [23] but displays at least one color signal that is highly visible against different backgrounds to the visual system of both conspecifics and a potential predator [23].

Some species of cleaning gobies have evolved yellow stripes against black (as has juvenile *L. bicolor*; Figure 2E). Here, colors used may depend on the specific substrate that gobies are viewed against, e.g., sponges. Gobies typically rest on the

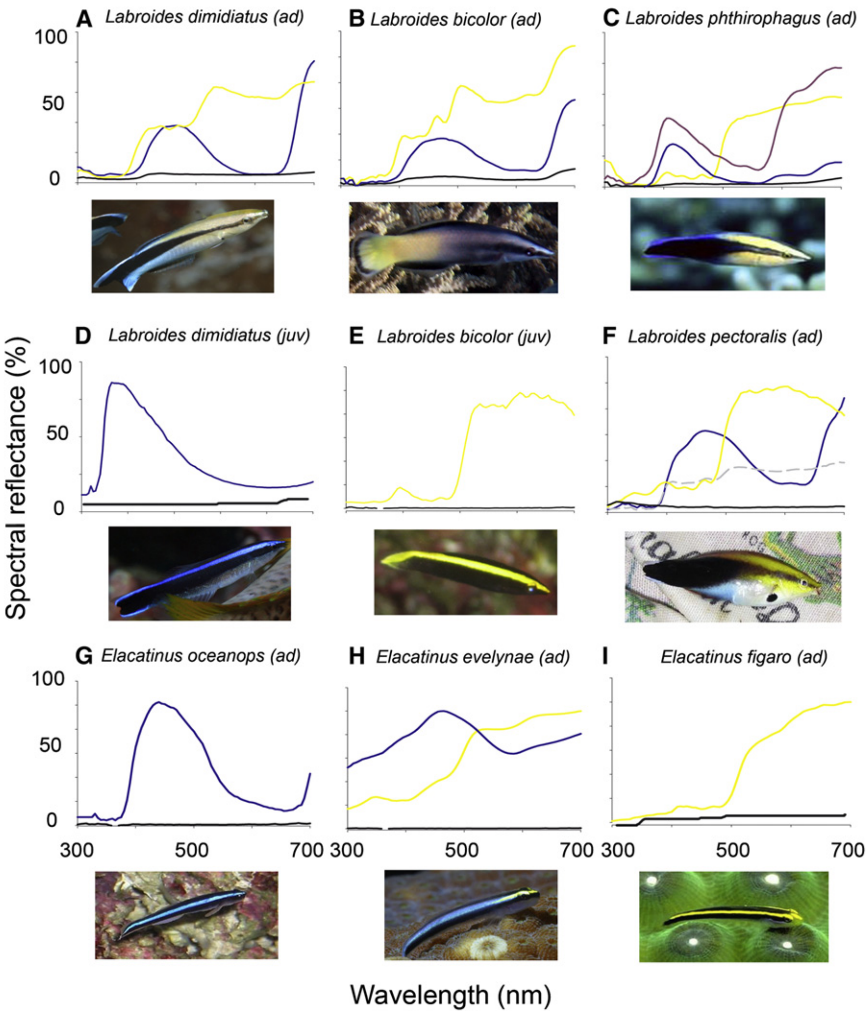

Figure 2. Spectral Distributions of Colors Measured on Each Fish Species

Curves are color coded to match photographs. ad, adult; juv, juvenile. Photographs by K.L.C.

The evolution of cleaner fish signals poses an interesting question: Which came first, cleaning behavior or signal coloration? Our study implies that perhaps cleaning behavior evolved first and signal coloration then followed as cleaners benefited from attracting more clients to cleaning stations based on their coloration. Facultative cleaners are often missing lateral stripes or a blue or yellow coloration (Table S1) but are still able to gain some of their dietary requirements from cleaning [1]. A subterminal mouth and small body size have also been suggested as prerequisites for cleaning [1, 2]; these features may enable cleaners to approach and remove ectoparasites from larger host fish.

Behavioral Experiment

In the field, we tested the response of wild client fish to seven fish models made from resin and painted with a range of colors and patterns (as per Figure 4). The first three models (Figures 4A–4C) were representative of adult *Labroides dimidiatus*. Model A was painted to be a true representation of *L. dimidiatus*, with the percentages of each colored area estimated from color photographs of *L. dimidiatus* (fish were photographed on the Great Barrier Reef). Blue was omitted from model B, and red replaced blue in model C. Model D represented the colors and patterns of a control fish, *Halichoeres melanurus*, which is of a size and shape similar to *L. dimidiatus* [16]; models E–G were painted different patterns to represent novel fish species. Models F and G had areas of blue, yellow, and black approximately equal to model A. The painted models were measured with a spectrometer, and colors were determined to be similar to those reflected from cleaner fish (Figure 2). The number and identity of fish that entered within 1 m proximity of the model, whether the fish approached the model, posed for the model [29], or expressed any other interest, were recorded. The number of visitors approaching each model ranged from 4 to 72 individual fish and from 2 to 30 species. There was a significant difference between models in the number of visitors (individuals range 4–72, $F_{6,66} = 11.30$, $p < 0.001$; species range 2–30, $F_{6,66} = 2.97$, $p = 0.02$), and model A was visited significantly more frequently than any of the other models (individuals: least squares difference [LSD] post hoc $p < 0.02$, Figure 4; species: LSD post hoc $p = 0.02$), including those with modified cleaner wrasse patterns (B and C). Fish models that contained blue (Figures 4A, 4D, 4F, and 4G), irrespective of pattern, were not more likely to attract fish to cleaning stations than other models ($F_{1,82} = 1.11$, $p = 0.29$). Unfortunately, because the aim of the study was to test whether cleaners had a blue

substrate before they approach clients because they are poor swimmers (K.L.C, unpublished data), unlike cleaner wrasses, which spend the majority of their time swimming in the water column above the coral reef (K.L.C. and A.S.G., unpublished data). Ancestral sponge-dwelling gobies also exhibit yellow stripes, whereas more recently derived cleaning species have evolved blue stripes [24]. How yellow gobies are viewed on a sponge background requires further investigation (but see [25]). Interestingly, some cleaning gobies (e.g., *Elacatinus evelynae*) have a yellow stripe that merges into blue (Figure 2H) and therefore also use a combination of blue and yellow.

The selective pressures that drive the evolution of visual signals should depend on the visual system of the signal receiver and the background against which it is viewed [26]. Plumage displays and ornaments, e.g. in bowerbirds, contrast with the visual background, and contrast has increased with the evolution of the bowerbird lineage in order for signals to be easily detected [27]. However, efficacy of signals depends upon within-pattern contrast in addition to background contrast [26, 28]. It is notable that yellow and blue patches on cleaner fish are often well spatially separated or contrasted with black. This results in a clear color pattern without spatial blurring of the colors, an effect possibly used in camouflage strategies in some other yellow and blue reef fish with finer yellow and blue markings [20] (see also Figure 4D).

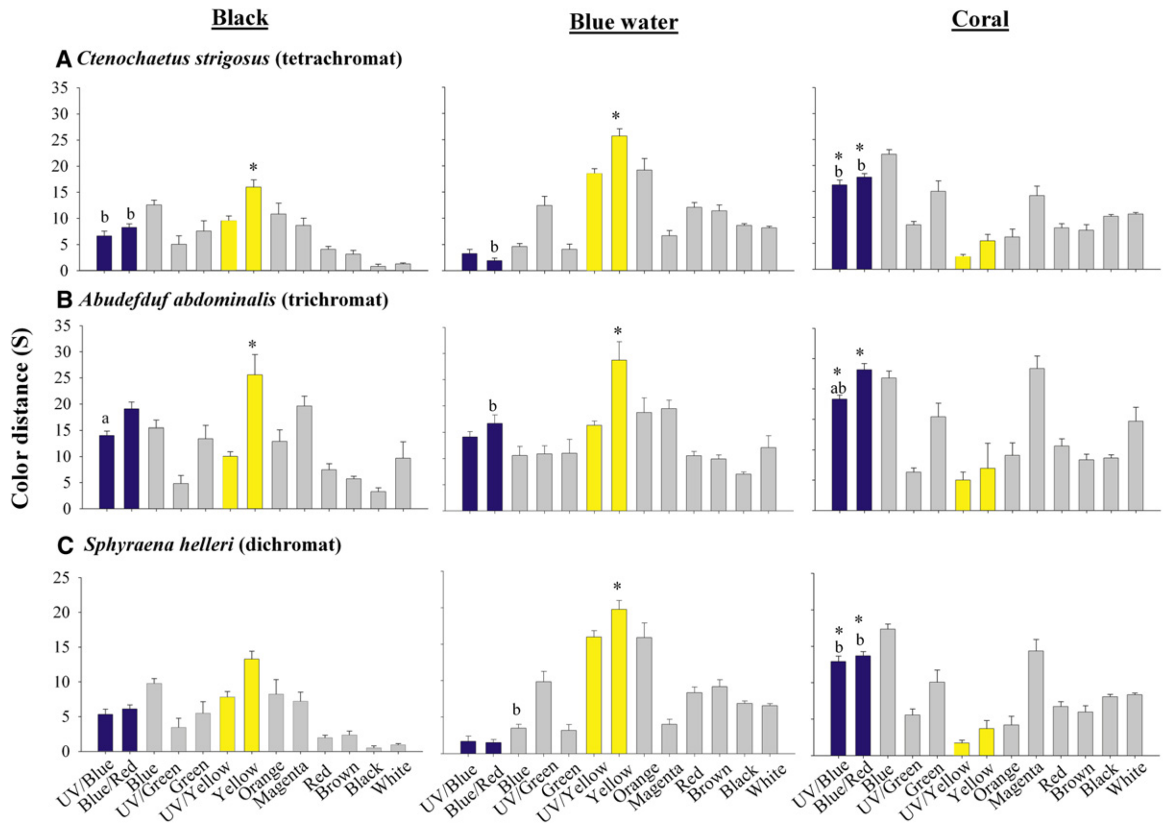

Figure 3. Mean Color Distance for Each Color Category against a Black, Blue Water, or Coral Background

Error bars indicate one standard error of the mean (SEM). Colored bars represent the color categories commonly found on obligate cleaner fish (UV/Blue, Blue/Red, Yellow, and UV/Yellow). *t > 2.10, $p < 0.05$ for indicated color category versus the average of the ten other categories; [a]$p < 0.05$ for UV/Blue versus Blue/Red; [b]$p < 0.05$ for UV/Blue or Blue/Red versus Blue.

guild coloration, the study was designed with 6 of 7 models having a yellow coloration. Therefore, we were unable to test whether yellow was also important in attracting fish to cleaning stations. However, both color and pattern appear to be important in cleaner fish signals.

We show that cleaners have evolved visual signals that are highly conspicuous colors to a wide range of signal receivers. Being conspicuous could be costly to cleaners if signal receivers were attracted for predatory purposes; however, cleaner fish are thought to be relatively immune from predation [1]. Color and pattern may therefore also communicate that clients should avoid eating cleaning organisms, thereby maintaining the mutualistic relationship between cleaner and client. Specific behavioral adaptations may also play a role in

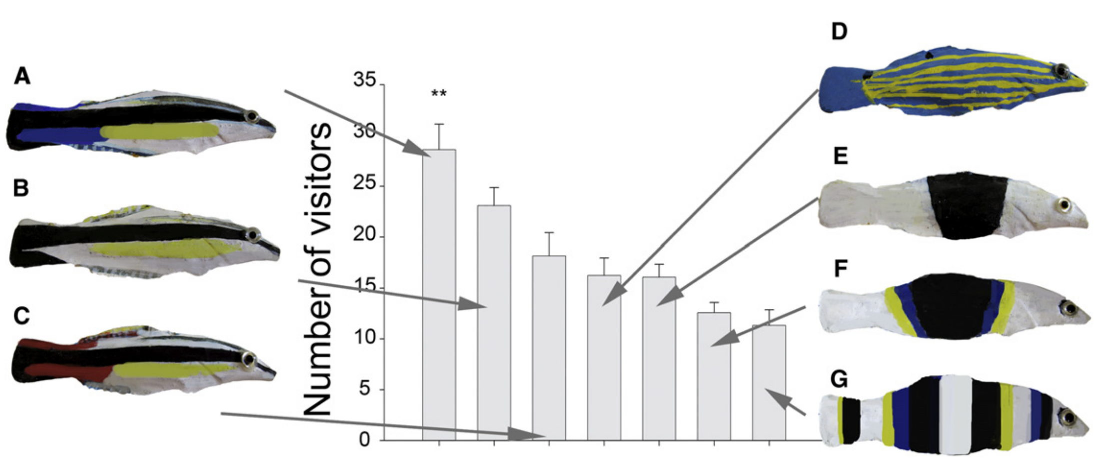

Figure 4. Mean Number of Individual Visitors to Each of the Replica Models per Ten Minutes

Error bars indicate one SEM. **$p < 0.05$ versus the other models.

advertising cleaning services; for example, cleaner wrasses frequently adopt an oscillating "dance" that is thought to attract fish to cleaning stations [30].

This is the first study to show that color is an important component in cleaner signaling. We have taken a comprehensive, nonsubjective approach to understanding the evolution and significance of color signals. Obligate and facultative cleaner fish were more likely to display a blue coloration compared to noncleaner fish, supporting the long-held belief that cleaners have evolved a blue guild coloration [5, 7, 31], but we have also demonstrated that yellow is important in cleaner signaling. A combination of color and pattern is an important component of cleaner signals and helps attract client species to cleaning stations [5, 7, 31]. This study attempts to understand the evolution of advertisement signals in a comparative context; this approach will be useful in future studies aimed at understanding colors used to signal information between animals or between animals and plants.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, two tables, and one figure and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01303-7.

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